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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY WASHINGTON, D.C. 20460

JUN 4 1991

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT:

Propiconazole. Accumulation of Tilt®3.6E in crayfish harvested from treated rice paddies. EPA Reg. No. 100-617. MRID Nos. 41819800 and 41819801. DEB No. 7843.

Barcode No. D162855.

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Tilt®3.6E is a broad spectrum fungicide registered for use on wheat, barley, rye, rice, sugarcane, pecans, and on grasses grown for seed (EPA Reg. No. 100-617). The active ingredient is propiconazole,1-{[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl}-1H-1,2,4-triazole. Permanent tolerances have been established for propiconazole and its metabolites determined as 2,4-dichlorobenzoic acid and expressed as the parent compound in several commodities including rice grain, 0.1 ppm, and rice straw, 3.0 ppm (40 CFR §180.434, 7/8/87). Propiconazole is a FIFRA 88 List C chemical. A registration standard for propiconazole has not been issued.

Ciba-Geigy requests an amended registration of Tilt®3.6E Fungicide to remove the restriction against polyculture of rice and crayfish in treated fields, and instead provide for a 220-day PHI between rice treatment and crayfish harvest. The petitioner has submitted a study monitoring the accumulation of propiconazole residues in crayfish and water resulting from treatment of rice paddies.

Conclusions

 The study treatment did not reflect the maximum amount of the a.i. applied to rice paddies at one time according to the proposed label.

- 2. Low residues of propiconazole were observed in the water collected from the Abbeville site following the second treatment of Tilt®3.6E. CBRS is concerned that the low residues were due to inadequate mixing and dispersion of the pesticide.
- 3. The nature of the residue in shellfish/crayfish is not understood. However, given the rapid depuration of residues in sunfish and the fact that the analytical method determines all compounds containing the 2,4-dichlorobenzoic acid moiety, a metabolism study in shellfish/crayfish will not be required unless residues are present in the viscera of crayfish harvested at the more practical PHI of 135-155 days (see conclusion 12 below).
- 4. a) The analytical method used to determine residues of propiconazole in crayfish is not adequate for enforcement purposes due to low and highly variable recoveries from crayfish meat and viscera.
 - b) The method called for the use of diazomethane, a dangerous reagent. If the petitioner intends to propose that the method be used for enforcement purposes, a justification for the use of diazomethane will be required.
- 5. The analytical method used to determine residues of propiconazole in water cannot be evaluated due to the modifications used, and a lack of representative chromatograms in water.
- 6. The petitioner did not adequately describe the processing of the crayfish into its edible meat and viscera portions. Quite often, the pancreas is packaged with the tail muscle as "fat." A detailed description of the components of the viscera will be required; all consumed tissues and byproducts should be included since residues concentrate in at least some components of viscera.
- 7. The treatment of crayfish prior to analysis was not adequately described; the petitioner did not indicate at what time the sample bags were composited.
- 8. The storage stability of propiconazole in crayfish meat and viscera is not known. The petitioner must demonstrate that residues are stable for (at least) the storage intervals used in this study.
- Storage intervals and analysis dates for crayfish samples harvested one day after treatment were not submitted with the petition.



- 10. Storage histories and data reporting sheets for water samples were not submitted.
- 11. Representative chromatograms for residues of propiconazole in crayfish were not submitted.
- 12. The 220-day PHI may not be practical, due to the fact that crayfish can be harvested as early as November. This would correspond to a PHI ranging from 135-155 days, if rice is planted on April 1. The petitioner should explore the possibility of carrying out a decline study to provide a basis for establishing a permanent tolerance for propiconazole in shellfish/crayfish. Alternatively, residue data supporting a PHI of 135-155 days must be submitted.

Recommendation

At this time, CBRS is unable to recommend in favor of removal of the crayfish/rice polyculture restriction on the Tilt®3.6E label and substitution with the proposed 220-day PHI for crayfish, based on the conclusions listed above.

DETAILED CONSIDERATIONS

Background

Tilt®3.6E Fungicide is an emulsifiable concentrate used to control sheath blight, brown leaf spot, narrow brown leaf spot, brown blotch, leaf smut, sheath spot, and stem rot in/on rice. According to the current label, Tilt® may be applied as an aerial spray at a rate of 0.17 to 0.28 lb a.i./A in 5 to 10 gallons of water. At the lower rate, two applications 10-14 days apart may be made, with the first application occurring at first internode elongation. The first internode elongation can occur anywhere from 25 to 42 days following planting. The higher rate is used for more pervasive infection, is also applied at first internode elongation, and should be followed by treatment with another registered fungicide if a second application is necessary.

The Sami Malak memo of 6/3/87 requested that the Tilt®3.6E label be amended to include the following notes under the rice treatment section:

- Do not use in rice fields where commercial farming of crayfish will be practiced.
- Do not drain water from treated rice fields into ponds used for commercial catfish farming.

DEB noted that residue data and proposed tolerances for residues of propiconazole in/on fish and shellfish would be required in order to remove the restrictions. Ciba-Geigy submitted a protocol for a



study of propiconazole residue accumulation in crayfish, which was reviewed by S. Malak, 6/27/88. The reviewer commented that an analytical method validation for water and crayfish would be required.

Rice grown in Louisiana represents approximately 18% of the total U.S. rice production (1984, Agricultural Statistics). In the state of Louisiana, anywhere from 20,000 to 37,000 acres of rice paddies are used for commercial polyculture of rice and crayfish. Approximately 65-100 million lbs. of raw crayfish are consumed in the U.S annually; more than 90% of the crayfish are grown in Louisiana. Up to 30% of the crayfish produced in Louisiana are the crayfish is approximately 15% of the total weight. Based on the higher figure for crayfish consumption, 100 million pounds annually, approximately 27 million pounds of raw crayfish are coultured in rice paddies, which corresponds to just over 4 million pounds of edible meat.

The commercial aquaculture of crayfish begins in April or May, when the adult crayfish are stocked into ponds; this step is deleted if crayfish are native or naturalized at sufficient populations. During May/June, the ponds are drained to force the crayfish to burrow into the mud. From June through August, a reflooded in October, and then the crayfish, including the adults and the larger young of both the native and stocked crayfish, are one third of the crayfish are harvested from November to February, late May or early June the cycle is started again. Crayfish in June.

Aquaculture in rice paddies follows a slightly different time scale. The paddies must be drained in late March or early April for rice planting. Crayfish are stocked (if not already sufficiently abundant) into the fields in June, when the paddies are flooded. In August the paddies are drained and the rice harvested. Paddies are generally reflooded in October, and the crayfish are harvested from November through April, when the cycle is repeated. The reflood, following the harvest of the rice grain, for the emerging crayfish. Crayfish production, providing forage with rice is expected to be higher in the fall and winter months, months.

Approximately 70% of the crayfish produced in the U.S. is consumed in the state of Louisiana, 50% of which is sold live to the consumer. The remaining 50% is sent to processing plants where it is parboiled, peeled, and the abdominal meat extracted and

packaged for sale. It is common practice to package the pancreas, referred to as fat, along with the meat in order to provide additional flavor during cooking; about 15% of the processed meat is sold without the pancreas. Packages of crayfish meat sold in supermarkets in Louisiana must contain no more than 8% fat by weight.

Experimental Design

The residue accumulation study was carried out by Wildlife International Ltd., Easton Maryland, for Ciba-Geigy Corporation. All residue analytical work was performed by Ciba-Geigy at the Greensboro North Carolina location. Physical and chemical properties of water and soil from the treatment and control sites were determined by A&L Southern Agricultural Laboratories in Pompano Beach Florida.

Two rice paddies located in parishes in south central Louisiana were chosen for the study. The Abbeville test paddy (AB-T) was located in the Vermilion Parish, and the Arnaudville test paddy (OP-T) was located in the St. Landry Parish. The Abbeville test paddy was 22 acres, and was flooded with water pumped from the Vermilion River. The Arnaudville test paddy was 4 acres, and was flooded with water pumped from the ground water table. Both test paddies were planted with Lemont rice (Oryza sativa L.). The test sites are sufficiently representative of important rice/crayfish treatment site (AB-C and OP-C), but the petitioner did not describe the exact locations and sizes.

Aerial applications of Tilt®3.6E were made to the Abbeville site on June 23 and July 16, 1988, and to the Arnaudville site on June 25 and July 14, 1988, at a rate of 0.169 lb a.i./A (5 gallons diluted product/A). Spray tanks contained the pesticide at a concentration of 1.2 oz. product/gallon. The petitioner claimed that the maximum label rate was used, yet the maximum registered label rate is still 0.28 lb a.i./A for more severe sheath blight; another registered fungicide if the disease persisted or reappeared. The study treatments represent the maximum label rate for one of two treatments with Tilt®3.6E. The study does not reflect the maximum label rate for Tilt®3.6E applied to rice paddies in one single application. The application equipment, calibration procedures, and application of the pesticide to the

Soil and water samples were collected from both treatment and control sites prior to and following applications of Tilt®. The pre-treatment samples were used to assess the physical and chemical characteristics of the test sites. Water samples were collected one day prior to treatment, immediately after treatment, and one, three, and five days following treatment. Soil and water were also



collected one day following the reflood in October. Control soil and water samples were collected prior to the first application, and one day following each application. Control water samples were spiked with known concentrations of propiconazole after collection to serve as analytical controls and validation samples. Adequate steps were taken to insure that sampling was random and representative. All samples were frozen immediately following collection, and shipped to A&L Southern Agricultural Labs.

Most of the crayfish were burrowed into the mud at the time of treatment; these were labelled the "native" crayfish. from neighboring ponds were collected in traps, and the traps were placed in the paddies prior to treatment with the fungicide. Samples of these "pre-stock" crayfish were collected by removing the traps and loading the crayfish into coolers. Native crayfish were caught in commercial traps which were baited and placed in the Arnaudville treatment site. Crayfish were collected prior to application, one day after both treatments, and then monthly beginning in February. Samples were collected until March in the Abbeville test plot, and until May in the Arnaudville plot. The typical crayfish samples contained 35-40 specimens; three samples were collected on each date. The sample sizes and locations in the test paddies were sufficiently random and representative; however, there was no indication of when the three samples were composited for analysis (i.e. prior to processing, prior to shipment, or prior to analysis). The petitioner stated that each sample contained 150 grams (0.4 lbs.) edible meat (tails with carapace).

Crayfish samples were processed and placed in the freezer within one hour of collection. If delays were necessary, samples were cooled on ice. The petitioner did not sufficiently describe the processing of the crayfish into its "edible meat" and "viscera" portions. Frozen samples were shipped overnight on dry ice to shipped in separate packages.

Nature of the Residue

The nature of the residue in plants and animals is adequately Residues of concern are the parent compound and metabolites determined as 2,4-dichlorobenzoic acid. The nature of the residue in fish and shellfish has not been elucidated. A fish accumulation study was reviewed by the EPA Environmental Assessment Accumulation propiconazole and elimination residues bioconcentration factor of 24X in muscle tissue; depuration was by nearly complete in 14 days. A shellfish/crayfish metabolism study will not be required unless residues are found to be present in the viscera of crayfish harvested at the lower PHI of 135-155 days (see conclusion 3, p. 2 of this review). to prohibit the use of water from treated fields in ponds used for The proposed label continues



commercial catfish farming. If the petitioner wishes to remove this restriction, a fish metabolism study will be required.

Analytical Methods

The analytical method used for water samples was "Gas Chromatographic Determination of Propiconazole and Etaconazole in Plant Material, Soil, and Water, " B. Buttler, J. Agric. Food Chem., Vol. 31, No. 4, 1983, in which residues are measured as the parent. Soil samples were not analyzed for residues of propiconazole. According to the method, water samples are partitioned into dichloromethane, and subjected to gas chromatography without further cleanup. Residues are detected with an alkali flame ionization detector in the nitrogen-sensitive mode. The published limit of detection is 0.001 ppm. The petitioner used a modified method, in which saturated sodium chloride was added to the water sample. The sample was then partitioned twice with 10% (v/v) ethyl Residues were determined as the parent using capillary gas chromatography with N/P detection. The petitioner did not submit representative chromatograms of propiconazole in water, therefore the method cannot be adequately evaluated.

The analytical method used to determine residues of propiconazole in crayfish meat and viscera samples was AG-517, "Determination of Total Residues of Propiconazole in Meat, Milk, and Eggs as 2,4-Dichlorobenzoic Acid by Capillary Gas Chromatography." According to the method, animal tissues are extracted by homogenization with 20% acetonitrile/water (15 g sample/200 ml solvent). The method was modified to use 15 g sample/150 ml solvent; the reason given was that samples were small in size, and that the smaller sample/solvent ratio allowed the analyst to retain some of the original sample. However, since each crayfish sample was to have contained approximately 150 g. edible meat, it is unclear why such an adjustment was necessary.

The extract is filtered, acidified, and evaporated to dryness. Potassium permanganate is added to the extract, along with sodium hydroxide, and refluxed for one hour and 15 minutes. During this step propiconazole and metabolites are converted to 2,4-dichlorobenzoic acid. The mixture is cooled, a small amount of water added, and then acidified prior to partitioning with 10% diethyl ether/hexane. The organic phase is then derivatized with diazomethane, cleaned up on an acidic alumina Sep-pak column, and analyzed by capillary gas chromatography and electron capture detection. The limit of quantitation (LOQ) in meat is 0.05 ppm. If the petitioner intends to propose use of this method for regulatory or enforcement purposes, the use of diazomethane, a dangerous reagent, must be eliminated or justified.

Other than the modification mentioned above, the petitioner did not mention any special sample treatments used for crayfish. It is unclear whether any additional processing prior to extraction

and analysis was necessary. point the sample bags were composited. Furthermore, it is unclear at what

Method Validation

Control water samples taken from the OP-C site one day prior to the second treatment were spiked with a standard solution of propiconazole at the time of collection. Residues of propiconazole were stable in the control samples. Analysis dates for water samples were not indicated, therefore the storage interval cannot be determined by the reviewer. Additional control water samples were spiked prior to extraction with a standard solution of propiconazole at 0.01, 0.02, 0.05, 0.10, and 0.20 ppm. Recoveries from water ranged from 63-129%, with a mean of 86%.

Crayfish pre-stocked into the paddies and collected one day prior to the first treatments were fortified prior to extraction with a standard solution of propiconazole at 0.05, 0.10, 0.20 and 0.50 ppm. Recoveries from meat and viscera were not expressed separately for these samples, and ranged from 42 to 84%, with an average of 64%. Native crayfish collected from the OP-T site one day prior to the second treatment with Tilt®3.6E were fortified with 0.20 and 1.0 ppm propiconazole, with an average recovery of

The meat and viscera of crayfish harvested after 2/20/89 were fortified separately. Meat was fortified with 0.05, 0.10, 0.20, 0.50, 1.0, and 5.0 ppm propiconazole. Recoveries from meat ranged from 40 to 90%, with an average of 62% (n=14). Viscera was fortified with 0.05, 0.10, 0.50, 1.0, and 5.0 ppm propiconazole. Recoveries from viscera ranged from 49 to 103%, with an average of

Average recoveries from both meat and viscera were low, and there was a high degree of variability. The petitioner did not provide any possible explanations for the low recoveries. Some of the fortified samples with low recoveries were repeated, usually with the same result. There were no trends in the data that could be linked to the LOQ. Recoveries of at least 70% are required in the Pesticide Assessment Guidelines, Subdivision O [Residue Chemistry, §171-4(b)]. CBRS might be willing to accept the method in crayfish if the petitioner provides a reasonable explanation for the low and highly variable recoveries from crayfish.

Storage Stability

Storage stability studies in crayfish meat and viscera were not submitted with this request. All crayfish samples harvested after 2/20/89 were analyzed within 41 days of collection; sample tracking for crayfish harvested after application of the pesticide was not adequate, and therefore the reviewer is unable to determine the storage interval for those samples. A stability study of



propiconazole residues in/on crayfish meat and viscera will be required.

Magnitude of the Residue

Residues of propiconazole in treated water decreased slightly in the first five days following application of the pesticide to the AB-T site. Prior to the second treatment, residues had decreased to 0.02 ppm. Residues decreased in a similar fashion at the OP-T site, resulting in <0.01 ppm propiconazole in the water samples collected prior to the second treatment. Following the reflood in October, both sites had residues of propiconazole <0.01 ppm.

Samples collected immediately following the second treatment at the AB-T site contained an average of only 0.02 ppm propiconazole; the samples were reanalyzed for confirmation, with the same result. The petitioner should provide an explanation for the low residues, given that the day-of-treatment residues for the remainder of the applications ranged from 0.07 to 0.10 ppm.

Representative chromatograms for residues of propiconazole in water were not submitted with the request. In addition, sample analysis dates and storage intervals were not included for water. It is possible that lack of storage stability resulted in the low residues measured in the T2 samples collected from the Abbeville test site following the second treatment. Residues of propiconazole in water are listed below.

Table I: Residues of Propiconazole in Water

Site		of)	Propiconazole	e in Water
	Study Day		Treatment	
Abbeville-C Abbeville-T	T1 - 1		(lb. a.i./A)	Propiconazole ^{b,c}
" " " " " " " " " " " " " " " " " " "	T1 - 1		Control Pre-treatment	<0.01
"	T1 + 3 T1 + 5		0.169	0.10 ~ 0.01 0.06 ~ 0.01
Abbeville-C	T2 + 1		# Comp.	0.08 - 0.01
Abbeville-T	T2 - 1 T2		Control Pre-treatment 0.169 + 0.169	<0.01
"	T2 + 3 T2 + 5 RF + 1		11 11	0.02 ~ 0.01 0.06 ~ 0.03 0.06 ~ 0.01
Arnaudville-C Arnaudville-T	T1 - 1	+	Control	<0.01, <0.01, <0.01
n n	T1 - 1 T1	P	re-treatment 0.169	<0.01
" Arnaudville-C	T1 + 3 T1 + 5	ļ	P1	0.10 ~ 0.05 0.04 ~ 0.01
Arnaudville-T	T2 + 1 T2 - 1	. Pr	Control 9-treatment	0.02 ~ 0.01 <0.01
11	T2 T2 + 3		169 + 0.169	<0.01 0.07 ~ 0.03
T = T	T2 + 5 RF + 1		**	0.03 ~ 0.01 0.05 ~ 0.01
treatment with	, RF = Reflood; Ti	1 + 1	= one day fo	<0.01, 0.01, <0.01 >11owing the 6

T = Treatment, RF = Reflood; T1 + 1 = one day following the first

Residues of propiconazole were concentrated in the viscera rather than in the edible meat of crayfish harvested one day following both treatments with Tilt@3.6E. representative chromatograms in crayfish meat and viscera were not included with the petition. Chromatograms were submitted for meat,



Each value is an average of three values for separate locations in the paddies, ~ standard deviation; reflood values were not averaged.

Residues are corrected for procedural recoveries <100%.

milk, and eggs, but cannot be considered as substitutes for crayfish. In addition, the method will need to be determined to be free of interference from other pesticides, since treatments with fungicides other than Tilt®3.6E are likely to occur in accordance with the label instructions.

The submission did not include any description of the viscera. Since the residues following treatment were found primarily in the viscera, CBRS is concerned that the pancreas, often packaged as "fat" with the edible meat, may contain residues of propiconazole. The petitioner will need to clarify what portions of the crayfish constituted the viscera, and address any residue concerns.

Crayfish harvested from February through May showed no accumulation of residues of propiconazole in either the edible meat The first February harvest was at 219 days, essentially the same as the requested 220-day PHI. The study reflects the proposed use, but the petitioner should have taken into account the fact that crayfish are generally harvested from commercial crayfish/rice polyculture as early as November. First internode elongation occurs approximately 25-42 days following planting. A November 1 harvest corresponds to a PHI of approximately 135-155 days, when rice is planted April 1, and two treatments at the lower application rate are used. The petitioner may want to propose a lower and more practical PHI, and submit data that would support a proposed tolerance for residues of propiconazole in shellfish/crayfish.

Residues of propiconazole in crayfish harvested one day after applications of Tilt®3.6E to rice paddies are shown below.

Table II: Residues of Propiconazole in Crayfish After Treatment with Tilt®3.6E

Site AB-T	B-T Mi		ppm Propi Me Range	conazole ^b at Mean ^d	ppm Propiconazole	
- "	T1 + 1	Pre-stocking	<.05-<.05	<.05	Range°	Meand
11	T2 + 1	.169 .169 + .169	<.0506	<.05	<.05 .4464	
OP-T	T1 - 1	Pre-stocking	<.0506	<.05	.4977	.51 ~ .1
"	T1 + 1	.169	<.05		<.05,<.05	.63 ~ .1
"	T2 + 1	.169 + .169	<.05-<.05	<.05	<.0531	<.05 .1413
<u>"</u>	T2 + 1	Mad 4	<.05,<.05	<.05	.2847	.36 ~ .08

T = Treatment; T1 + 1 = one day following the first treatment with <math>Tilt = 3.6E.

Residues were determined as 2,4-Dichlorobenzoic Acid and converted to

equivalents of propiconazole.

The submission did not include sample histories for the crayfish analyzed to produce the above table, and analytical worksheets were not included. There is no way to ascertain the time interval between sample processing and analysis. Residues tended to concentrate in the viscera immediately following treatment of the paddies.

Since propiconazole is relatively nonpolar, it is possible that residues could accumulate in the viscera due to prolonged exposure. The petitioner may want to consider performing a decline study. This should permit the establishment of a proper tolerance and PHI that more accurately and simultaneously reflect the variables of aquatic dissipation, crayfish accumulation and depuration of residues, crayfish/rice cultural practices, and the practicality of the proposed PHI.

Residue values for crayfish harvested after the proposed 220-day PHI are listed below.

Table III: Propiconazole Residues in Crayfish After 220 Days

Site	Treatment (lb a.i./A)	Sample	PHI	ppm Propiconazole*	
AB-T	.169 + .169	Sample Date	(days)	Meat	<u>Viscera l</u>
17		2/20/89	219	<0.05	<0.05
11	Н	3/10/89	237	<0.05	
<u> </u>	#	3/29/89	256		<0.05
OP-T	.169 + .169	2/20/89		<0.05	<0.05
	11		221	<0.05	<0.05
н	"	3/10/89	239	<0.05	<0.05
11	"	3/29/89	258	<0.05	<0.05
11		4/17/89	277	<0.05	
	11	5/30/89	320	<0.05	<0.05 <0.05

Determined as 2,4-Dichlorobenzoic acid and converted to equivalents of propiconazole.



c Residue values were corrected for procedural recoveries <100%.

For samples reported as containing <.05 ppm propiconazole (that is, less than the LOQ), a value of 0.025 ppm, or half the LOQ was used to calculate means. Each sample was comprised of three replicates; meat replicates from the AB-T site, and viscera replicates from the OP-T site were analyzed twice.

[°] Crayfish native to the site were sampled; all other crayfish samples were those pre-stocked into the paddie in traps prior to treatment.

Each value represents a mean of three replicate determinations.

The quality of the representative chromatograms are examined and found to be free of data matrix interferences, interfering peaks, and background noise. addition, the treatments did not reflect the maximum label rate for a single application of Tilt®. Furthermore, even though residues remaining in crayfish after 220 days were negligible, the practicality of the 220-day PHI is questionable, since crayfish may be harvested during November (130-day PHI in the subject study). The components of the viscera were not adequately described; CBRS is specifically interested in the pancreas, which can be packaged with the meat. Any tolerance proposals for propiconazole in crayfish submitted by ICI Americas should distinguish between acceptable residue levels in meat and viscera.

Meat, Milk, Eggs, and Poultry

The commercial crayfish industry is continually searching for means to increase the economic yield from the crop. It has been reported that a crayfish waste-processing company in Louisiana is producing fertilizer supplements, animal feed meals, and pigment-rich oils from crayfish processing waste ("Crawfish Culture in the Southeastern USA, J.V. Huner and R.P. Romaire, World Triculture, 21(4), pp. 58-65, 1990). However, crayfish and processed crayfish The proposed amended use is not expected to result in increased residues of propiconazole in meat and meat by-products, eggs, and poultry.

cc: CBSwartz (CBRS), C. Furlow (PIB/FOD), FIFRA 88 List C File, Circulate (7), RF, Tilt SF, Propiconazole SF

H7509C:CBRS:CBSwartz:CM#2:Rm 800D:703-557-1877:4/30/90 RDI: WJHazel: 5/21/91 EZager: 5/31/91